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WHEN TO QUIT? ESTIMATING SPIDER SPECIES RICHNESS IN A NORTHERN EUROPEAN DECIDUOUS FOREST

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ABSTRACT. Terrestrial arthropod surveys and inventories frequently suffer from undersampling bias; common species are over-represented and rare species may be missed entirely. This study compared a rapid (3 days) and intense inventory of spiders from one hectare of a mature beech forest (*Fagus sylvaticus*) in Hestehaven, Denmark, comprising 8,710 adult spiders of 66 species to a previous, much more thorough, bi-weekly survey of two years duration from the same site that comprised 42,273 spiders (adult and juvenile) of 141 species. Non-parametric species richness estimators were used to assess the degree of undersampling bias in various data partitions. The current study used five experienced, four novice collectors, and five semi-quantitative collecting methods. Method and time of day strongly affected numbers of species and adults per sample. Collector experience affected numbers of species but not numbers of adults per sample. Despite the intensive collecting, number of adults per sample did not decrease over the course of the study. At the end of the sampling, 31 species were still rare in the sample (singletons or doubletons). Non-parametric richness estimators suggest that the actual richness of adult spiders in the study plot at this time of year and susceptible to the methods used was about 80 species. Species turnover between the two surveys (ca 23 years) was remarkably small: the two lists were 92% identical. The baseline study suggests that the rarity of 12 of the 31 rare species was artifactual (10 due to phenology, one to method, another to spatial edge effects). The rarity of the remainder is unexplained and by default is interpreted as undersampling bias.

Keywords: Biodiversity, Araneae, inventory, species richness estimation, singletons, beech forest, Denmark

Conservation and natural resource management decisions notoriously draw mainly on ecological information obtained from vertebrates and plants (Kremen et al. 1993; Prendergast et al. 1993). Environmental monitoring is much the same, even though it is widely recognized that patterns in vertebrates and plants do not exemplify the patterns in many other groups in the same habitat (Groombridge 1992; Stork & Samways 1995). The bias towards vertebrates and woody plants stems from a simple reason: surveys are usually so short and resource-limited that only the best-known and least diverse groups can be adequately sampled.

One consequence is that arthropods, the most diverse organisms in any terrestrial environment (May 1978), often go unstudied (Longino 1994). The most basic data provided by traditional biodiversity assessments is the richness and relative abundance of species in a given area (May 1975; Taylor 1978) and on both counts arthropods present formidable challenges (Erwin 1983; McKamey 1999; Novotny & Basset 2000). Arthropods, however, may provide information not otherwise obtainable from traditional focal groups; information that may turn out to be crucial for long term management of existing natural resources (Kremen et al. 1993). They are small in size (therefore abundant), short-lived (going through many generations within short time spans), diverse and often have limited distributions and strict environmental requirements (e.g., many mites and small spiders live exclusively within a few square meters of soil on the forest floor). In theory, they should map environmental diversity and track environmental changes more quickly and precisely than longer-lived, more flexible organisms such as vertebrates and plants.

Collecting prodigious numbers of species and individuals is easy, but the proportion of the total available fauna represented in such collections is usually unknown. The omnipresent high frequency of rare species instead suggests that arthropod communities in general are drastically under-sampled by conventional survey efforts, even large ones. To judge the real utility of arthropods for environmental assessment and monitoring, therefore, we first need to be able to assess the thoroughness and efficiency of inventories and censuses themselves. These, in turn, require relatively fast, cheap, efficient and robust sampling protocols. Such methods have been proposed for spiders in tropical ecosystems by Coddington and co-workers (Coddington et al. 1991) and tested in Cameroon, Tanzania (Sørensen et al. 2002), Madagascar, Bolivia (Coddington et al. 1991, 1996), Guyana, Tobago, southern USA (Coddington et al. 1996; Dobyns 1997), Slovenia (Kuntner & Baxter 1997), Denmark (this study) and Greenland (Larsen & Rasmussen 1999).

The optimal test of empirical and analytical inventory methods would be against a "known universe" in which the fauna is a) natural, b) diverse, and c) thoroughly known. For spiders such sites are few indeed. One possibility is the International Biological Project (IBP) site (Thamdrup et al. 1975) in Hestehaven, Denmark. The information available on spiders from this mature beech forest is unique because collecting was carried out biweekly for several years in the 1970's with a large battery of ecological sampling methods. All material collected was identified to species and instar, including juveniles (Toft 1976). The accuracy of juvenile identifications is frequently, and perhaps justifiably, questioned. Toft (1976) argued that in his specific case the error rate was acceptably low because the somatic morphology of many species was distinct, the total diversity was low and the clarity of distinct phenologies resulting in unambiguous adults made retroactive identification of juveniles feasible (see also Toft 1983). Because even crude measures of the effect of ignoring juveniles are almost nonexistent in the literature (but see Norris 1999) and because the question is intrinsically interesting, for the purposes of comparison to our data we accept the accuracy of Toft's determinations. Data from identified juveniles reveal community phenology patterns, and also can quantify the bias resulting from the practical necessity of modern inventories to focus on adult animals only, whether in tropical inventories or less well understood temperate areas. The proportion of juveniles in a tropical sample seems remarkably constant at 60–70% (Coddington, Scharff, pers. obs; Russell-Smith & Stork 1995; Silva 1996). Tropical assessments to date have worked with adults only, because tropical spider faunas are so little known that identification of juveniles of other than ostentatious species is impossible. Even adults are often impossible to identify to anything but morphospecies in the tropics. The main objective of this study was to evaluate in several ways this inventory design against a "known spider universe." Of course, local faunas do change with time (in this case a 23 year hiatus) and stochastically due to dispersal and local extinctions. Nevertheless, we know of no spider communities from climax communities as well known as that of Hestehaven. We wanted to test how well the method worked in a temperate forest community where a few species would be numerically dominant and wished to investigate the impact of extremely rapid surveys using many simultaneous collectors (with a mix of professional arachnologists and collectors with little or no collecting experience) on the fauna, and to learn how unavoidable factors such as method, day vs. night collecting and collector experience affected results. Finally we wished to calibrate richness statistics from an extremely rapid and intense inventory against the known richness for the same season and against the total known annual spider fauna.

METHODS

Study site.—The study was carried out during 3 days, August 29–September 1, 1994, in the mixed coastal forest, Hestehaven (176 hectares), about 25 km NNE of Århus, Eastern Jutland, Denmark. The forest is approximately 15 meters above sea level and surrounded by agricultural land. A one hectare sampling plot

(56°17.46′N, 10°28.50′E) was established within a 3-hectare climax stand of mature beech (*Fagus silvaticus* L.). A map of the forest including the location of the sampling plot and the distribution of vegetation is given in Rasmussen et al. (1982; fig. 2). The nearest stand of non-beech vegetation is located approximately 75 meters from the sampling plot and consists of spruce. The distance from the sampling plot to the nearest agricultural area is 250 meters. The plot perimeter was marked with strings to ensure that all collecting only took place within the plot. Danish ecologists have intensively studied the arthropod fauna of this particular stand of beech in the period 1969 to 1972 in connection with an international study of beech-wood ecosystems (Nielsen 1974a, 1974b, 1974c, 1975, 1977, 1978a, 1978b, 1987). The composition of the spider fauna was analyzed by Toft (1976, 1978) and other scientific results of this beech-wood project in Hestehaven have been published in 66 scientific papers. There is no other place in Denmark, and few elsewhere in the world, where a well-known arthropod fauna has been studied in such detail.

The oldest beech trees within the plot are more than 110 years old and very little regeneration occurs. The density of beech trees is approximately 190 trees per hectare. Mature beech forests severely reduce light reaching the forest floor, and their root systems effectively compete against other woody plants. The Hestehaven forest floor vegetation is dominated by *Anemone nemorosa* L., *Melica uniflora* Retz., *Asperula odorata* L., *Hordeum europaeum* (L.), *Circaea lutetiana* L., *Carex sylvatica* Huds., *Veronica montana* L., and *Ficaria verna* Huds., (Nielsen 1977). At the end of August, the forest floor was dominated by knee-high grass and scattered areas with ferns. As is typical for mature northern European beech forests, the understory supported few bushes and small trees and therefore very little vegetation that could be reached by hand.

Collectors.—Nine collectors worked simultaneously in the field. Five of these were classified *a priori* as "experienced" (many years collecting spiders), and the other four as inexperienced (no or less than one year experience in spider collecting). Sampling began on August 29 at night (2000–2400), continued day (0900–1800) and night on August 30–31, and concluded during the morning of September 1. Even by "rapid" inventory standards three days is extremely short, but various scheduling conflicts prohibited a longer duration. Collecting both night and day is important to make sure that both diurnal and nocturnal species are collected. Each collector was asked to use each collecting method a certain number of times during the fieldwork. Collectors were limited to 6 or fewer samples per day or night to avoid fatigue. One person kept track of all the samples taken by various collectors, methods and times of day, thereby ensuring that the different methods were used both day and night and making sure that work was carried out efficiently.

Collecting methods.—We used five collecting methods to access the spider fauna within the plot. These were chosen to access as many different habitats, and to overlap as little as possible. Because the time span of the inventory was so short we did not use pitfalls and for logistic reasons Berlese or Tullgren funnel extraction of litter were not feasible. Each sample represented one method applied for 1 hour of active, continuous collecting (i.e., including time required to transfer the catch to a vial, but excluding time due to interruptions). Collectors used countdown time functions in wristwatches to time themselves. The countdown was suspended if the collector moved to a new habitat patch or if occupied with non-collecting tasks (i.e., logistics, equipment maintenance, field notes, photography, etc.). A sample therefore usually took somewhat more than one hour to finish.

Aerial: Searching through the vegetation from knee height to as high as the collector can reach above his/her head. Toti et al. (2000) changed the name to "aerial" to emphasize the target guild, but it is synonymous with "looking up" method of Coddington et al. (1991).

Ground: Searching the ground and lower vegetation below knee height. Toti et al. (2000) changed the name to "ground" to emphasize the target guild, but it is synonymous with "looking down" method of Coddington et al. (1991). The collector searches on hands and knees for spiders on the surface of plants, tree stems, logs, rocks, and the ground surface but not the interior of leaf litter, logs, under stones etc.

Sweeping: Searching the lower herb layer with a sweep net (net diameter 36 cm). The net was emptied after a few sweeps to avoid damage to the specimens. In this study the diversity of the vegetation available for sweeping was rather limited and dominated by grass and small, scattered areas with ferns.

Beating: Sharply tapping branches or other vegetation with a stout stick while holding a 0.6 m^2 beating tray underneath to catch the falling spiders. Beating tray areas varied among collectors, but because samples were defined on the basis of time rather than repetitions or area, beating tray areas are probably unimportant. Small spiders are efficiently transferred from the beating tray to the sample vial with an aspirator or pooter. Because mature beech trees have very few lower branches, this plot had little vegetation suitable for beating, and consequently we allocated fewer resources to beating and more resources to other methods. Beating at night was difficult because of headlamp glare and yielded sparse results, so we eliminated that combination.

Cryptic: Searching for adult spiders under logs, inside rotten logs, sifting litter, manual search within leaf litter, under rocks, inside holes, under bark, etc. It is intended to access any habitat the "cryptic" fauna is likely to occupy and allows the collector to use the method best suited to the opportunities the particular habitat offers.

Specimens and sorting procedures.—Each sample was labeled with locality, date, collector, method, and replicate number (if two samples were otherwise identical). Samples were more or less immediately transferred to 70% ethanol in a WhirlPak® bag so that field vials could be reused. A mixture of experienced and inexperienced (students) arachnologists working in groups sorted the collection to species so that the experienced arachnologists could validate identifications (identifiers are listed in the Acknowledgments). All identifications of singletons and doubletons were checked and verified by several arachnologists. Voucher specimens of each species identified in this study are deposited at the Zoological Museum, University of Copenhagen (ZMUC). Duplicates have been deposited at the Smithsonian Institution, Washington D.C. (USNM) and at the California Academy of Sciences (CAS), San Francisco, CA.

Statistical analysis.—Statistical analyses and graphs were produced with Systat 9.0 (SPSS Inc. 1999). To analyze the effects of inventory design parameters on results, we chose analysis of variance in which method, time of day, and collector experience were treated as independent factors, and numbers of adults and species per sample, respectively, as dependent variables. Post-hoc Tukey HSD tests were used to determine which treatments were responsible for significantly different factors. Due to the large number of factors and treatments, some ANOVA cells were empty. For example, we did not beat at night, and therefore beating was excluded from analyses involving method and time of day. A third analysis investigated the influence of individual collectors on the overall mean number of species per sample. A fourth analysis contrasted the number of species per sample by method and time of day for sets of experienced and inexperienced collectors. Species accumulation curves and richness estimates were produced with EstimateS 6.0b1 (Colwell 2000). The current dataset is hereafter referred to as "ZMUC" (Zoological Museum University of Copenhagen) and the historical dataset from Toft (1976) is referred to as "AAU" (Aarhus University).

Lognormal distributions were computed manually as no available programs retain the benefits of the classical approach and also solve the problem of the biased 0–1 octave (Lobo & Favila 1999; Longino et al. 2002). Many programs define abundance classes as log base 3, which prevents integer values from falling on class boundaries, but it also collapses the full distribution to relatively few abundance classes for most datasets, and the chi square test therefore lacks power. Log base 2, as Preston (1948) originally suggested, maintains a relatively fine-grained classification of the data and is easy to compute. The problem of singleton species is more subtle. Most techniques (e.g. Preston 1948, 1962; Ludwig & Reynolds 1988; see also Bliss 1966) apparently divide the singleton species between the 0.5–1 and 1–2 octave, just as other values falling on class boundaries are divided. However, all higher octaves potentially receive from both neighboring boundaries, but the 0.5–1 octave cannot draw from the 0.25–0.5 octave, as species with fractional relative abundances are not observed. The practical effect of this bias is that the 1–2 octave is always larger than the 0.5–1 octave because it contains half the 1's and 2's. This produces a false mode in the data and distorts the calculation of the lognormal parameters. Because the 0.5–1 octave is always biased, it should be ignored during the calculation of parameters. We iteratively assigned "octave" numbers (r) to the log base 2 abundance classes and estimated the lognormal parameters S0 (mode) and a (width) using the Nonlin module of Systat 5.2, with Quasi-Newton estimation and least squares fit (model: $S = S_0 e^{(-a2 \cdot r2)}$. The optimal set of assignments minimized the chi square difference between estimated and observed richness (s) across octaves.

"Sampling intensity" is the ratio of specimens to species (Coddington et al. 1996; Sørensen et al. 2002). The chief virtue of this measure is its simplicity: it can be calculated for any inventory whatever. Given roughly comparable relative abundance distributions and richness, it crudely compares sampling effort to the size of the universe being sampled (but see Gotelli & Colwell (2001) for pitfalls). Inventory completion (or completeness) is the extent to which an inventory, or inventory component, samples the faunal partition available to it (Sørensen et al. 2002). Equal sampling effort in microhabitats or diversity partitions that vary in richness can result in disproportionately rich microhabitats being disproportionately undersampled. The usual symptom of such biased sampling is a strong correlation between sampling effort and richness (Heyer et al. 1999), which in turn can bias conclusions about relative species richness. For spiders, different methods and day versus night collecting access different partitions of the overall community with varying efficiency, and those partitions also differ in richness and abundance (Silva 1996; Silva & Coddington 1996; Coddington et al. 1996; Sørensen et al. 2002). We measure "inventory completion" in an inventory partition as the ratio of observed richness to the Chao1 richness estimate for that partition (Sørensen et al. 2002). Comparison of species richness estimators generally favor Chao2 as among the least biased, most efficient, and most robust methods (Colwell & Coddington 1994; Peterson & Slade 1998; Walter & Martin 2001). Chao2, however, requires replicate sampling. Chao1 performed nearly as well as Chao2 in tests, is simply calculated from tabular data, and is the only non-parametric richness estimator that does not require replicate sampling. It can therefore be applied to more kinds and qualities of inventory data, and will enable broader comparison of completion statistics across inventories. Our allocation of sampling effort reflected the idiosyncrasies of the site and our *a priori* assessment of the relative richness of different microhabitats. The dense beech canopy had suppressed nearly all understory shrubs and the beech trees themselves lacked all lower branches. The herb layer was knee-high uniform grass with interspersed fern clones. Therefore, we allocated relatively less effort to beating and more to cryptic and ground searching compared to aerial searching and sweeping. Ideally, an inventory should be an unbiased sample of the community. In practical terms this means that each method or time of day partition should reach the same degree of inventory completion; equivalently, the coefficient of variation of inventory completion should be equal to or less than that of sampling effort investment across the variation inventory partitions.

RESULTS

The nine collectors produced 149 samples over 3 days containing a total of 8,710 adults of 66 species from the one hectare plot (Appendix; Table 1). The mean number and standard deviation of total samples per collector was 16.56 ± 0.72 (n = 149), aerial was 2.33 ± 0.71 (n = 21), beating was 0.89 ± 0.71 (n = 8), cryptic was 5.56 ± 1.59 (n = 50), ground was 5.22 ± 1.72 (n = 47) and sweeping was 2.56 ± 1.01 (n = 23). Overall sample intensity (specimens : species) was 132, but it ranged from 24–110 per method (because methods often catch the same species, the total sample intensity is usually greater than that of any partition). The figure of 132 may seem high, but is biased by the extraordinary abundance of two species (*Linyphia triangularis* (Clerck 1757) and *Drapetisca socialis* (Sundevall 1833)). If these are excluded, the ZMUC sampling intensity falls to the mediocre value of 12, which is well below 30, our current working guess of the minimum sampling intensity statistic typically sufficient to yield convincingly asymptotic richness estimates. Nineteen species were singletons and 12 were doubletons. Despite the large number of animals collected, the final percentage of singletons was high at 29%. *Linyphia trian-*

Table 1.—Summary values for the ZMUC inventory at Hestehaven. SD = Standard deviation, Spp = Species. Percent method bias is the deviation of the method's inventory completion statistic from the grand mean among methods. Percent effort investment is the percent of total samples invested in a particular method.

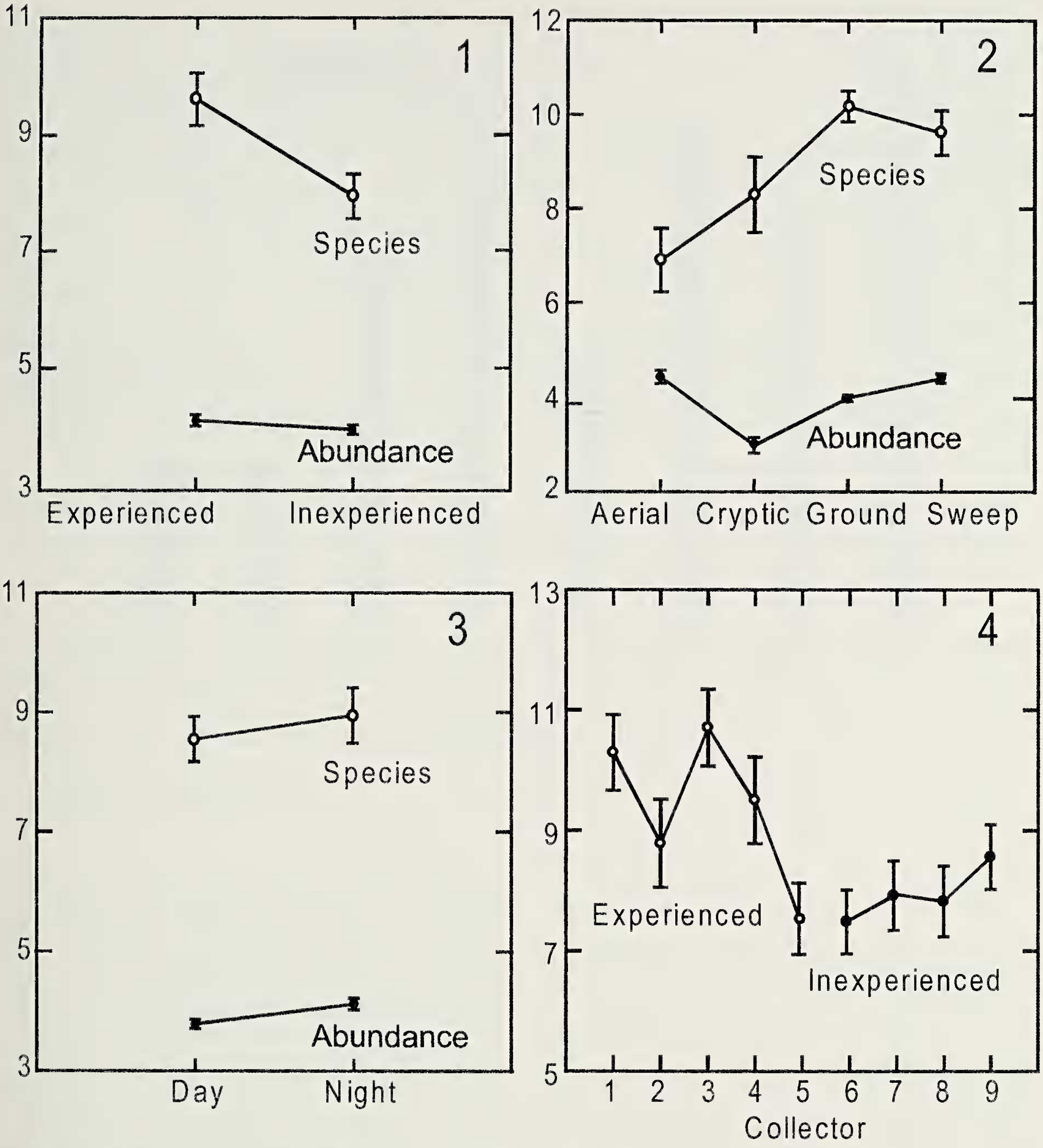
	Collection methods					Time		
	Aerial	Beating	Sweeping	Ground	Cryptic	Day	Night	Total
No. of samples	21	8	23	47	50	96	53	149
Mean no. of ind./sample	120	51	92	58	19	41	90	58
SD ind./sample	72	29	44	17	9	34	9	49
Mean no. of spp./sample	7	7	10	10	8	9	9	9
SD spp./sample	2	2	2	2	3	3	3	3
Total no. of individuals	2,526	405	2,126	2,713	940	3,960	4,750	8,710
Total no. of species	23	17	30	38	34	53	44	66
Sample intensity	110	24	71	71	28	75	108	132
No. of unique species	2	1	9	9	9	22	13	30
Singletons	5	5	10	13	7	17	13	19
Doubletons	4	0	2	3	4	7	5	12
% Singletons	22	29	33	34	21	32	30	29
Chao1 estimate	26 ± 3	25 ± 8	55 ± 16	66 ± 16	40 ± 5	74 ± 13	61 ± 13	81 ± 7
% Inventory Completion	88	68	55	58	85	72	72	81
% Method Bias	17	−3	−16	−13	14	1	1	10
% Effort Investment	14	5	15	3	34	64	36	100

gularis and *D. socialis* at 2,135 and 2,046 individuals, respectively, dwarfed the abundances of other species and accounted for 48% of the total inventory. The true relative abundance of at least *D. socialis* was even greater because we truncated collection of this species at 10 specimens per sample after the first night. We continued to collect *L. triangularis* because it could not be reliably distinguished in the field from the much rarer *Linyphia hortensis* Sundevall 1830 or *Neriene clathrata* (Sundevall 1830). Hourly samples averaged 58 individuals and 9 species overall. Cryptic sampling yielded the fewest individuals per hour (19) and aerial the most (120), but methods were remarkably uniform in average numbers of species per sample (7–10). Richness per sample ranged from 2–14 species, and abundance from 2–273 individuals.

Collector experience, method, and time of day.—Abundance but not number of species per sample required log-transformation prior to analysis to maintain normality. Collector experience, method, and time of day were treated as independent factors in the ANOVA model, and numbers of adults and species per sample, respectively, as dependent variables. As mentioned under "Methods," beating at night is difficult due to glare and at this site was unproductive. We therefore excluded beating at night as a method-time of day combination and excluded it from these analyses. Collector experience significantly increased number of species per sample, but not number of adults ($F = 7.029$, $P < 0.000$, Fig. 1). Method affected both number of species ($F = 7.029$, $P < 0.000$) and numbers of adults per sample ($F = 20.429$, $P < 0.000$, Fig. 2). Aerial and sweep sampling produced more adults per sample than cryptic ($P < 0.000$ vs aerial; $P < 0.000$ vs. sweeping) or ground sampling ($P < 0.026$ vs aerial; $P < 0.003$ vs. sweeping), and cryptic and ground also differed significantly from each other ($P < 0.000$). For numbers of species per sample, sweeping, ground and cryptic collecting did not differ from each other (Fig. 2), but aerial produced fewer species per sample than ground ($P < 0.000$) or sweeping ($P < 0.006$). Night collecting significantly increased numbers of adults per sample ($P < 0.009$) but not species (Fig. 3). The model explained 75% of the variance in numbers of adults and 34% of the variance in numbers of species per sample. No factor interactions were significant in either ANOVA. To investigate more fully the effect of individual collectors on numbers of species per sample, we ran an ANOVA with collector identity and method as independent factors and numbers of species per sample as the dependent variable (Fig. 4). A post-hoc Tukey HSD test showed that collectors 5 and 6 differed from 1 and 3, and collectors 7 and 8 also differed from 3. Collector 5, classified a priori as experienced was more similar to inexperienced collectors (collector 5, Fig. 4). During the day experienced collectors were much more efficient at aerial searching and beating, less so at cryptic and ground searching, and indistinguishable from inexperienced collectors at sweeping (Fig. 5). At night inexperienced collectors were only slightly less efficient at aerial sampling and were equivalent sweepers, but the gap widened during cryptic and ground collecting (Fig. 6). Sweeping was the only method used here that seemed completely unaffected by experience. In summary, method strongly affected both abundance and richness, experience produced moderately larger numbers of species but not individuals and spiders were generally more accessible (active) at night than during the day.

Complementarity of methods.—Thirty species were unique to single methods and the overlap between methods was moderate, ranging from 11 species shared between "ground" and "beating" to 22 species shared between cryptic and ground. Each method sampled unique species not found by the other methods (Table 1).

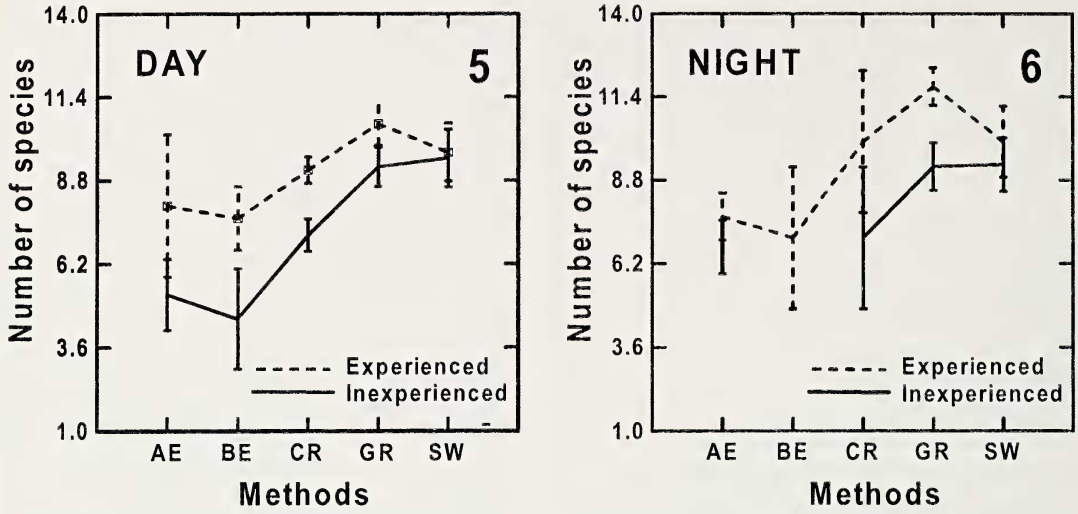
Faunal depletion.—We tested for the effect of intensive collecting on the overall spider fauna by plotting individuals per sample against chronologically arranged sample number (Fig. 7). If all species are included, abundance does decrease over the sampling period ("All species," Fig. 7). However, this decrease is primarily due to our decision after the first night to truncate collection of the very abundant *D. socialis* in each sample after 10 animals had been collected. If the two most common species are excluded (*D. socialis*, *L. triangularis*), spider abundance per sample did not decrease significantly over the course of the study ("most common excluded," Fig. 7). We further checked this result by lagging the data and testing for cross-correlation to the



Figures 1–4.—Least squares means and standard errors from analysis of variance on number of species (open circles) and log of numbers of adults (closed circles) per: 1. Sample by collector experience. Experience increases richness but not abundance per sample; 2. Sample by method. For abundance, cryptic and ground sampling differ from each other and both from aerial and sweeping. For richness, aerial differs from ground and sweeping; 3. Sample by time of day. Night collecting increases number of adults but not species; 4. Least squares means and standard errors from analysis of variance on number of species per sample by experienced (open circles) and inexperienced (closed circles) collectors.

original time line; the plot showed no significant trend. Collector fatigue and boredom with common species probably also played a role.

Inventory completion.—The mean inventory completion by method was 71%, and separate methods deviated −16% to +17% around this value (percent method bias, Table 1). Figure 8 compares observed to estimated richness for each method, day versus night, and the total inventory. Aerial sampling was most complete at 88% and sweeping least



Figures 5–6.—Least squares means and standard errors from analysis of variance on number of species per sample by method and collector experience during: 5. Day; 6. Night (Abbreviations: AE = Aerial, BE = Beating, CR = Cryptic, GR = Ground, SW = Sweeping).

complete at 55% despite essentially equal sampling effort. Day and night sampling, on the other hand, were equally complete at 72%, despite nearly twice as much investment in daytime sampling (Table 1). Overall, the coefficient of variation for sampling effort across methods and times of day was 68%, but that for inventory completion only 18%, showing

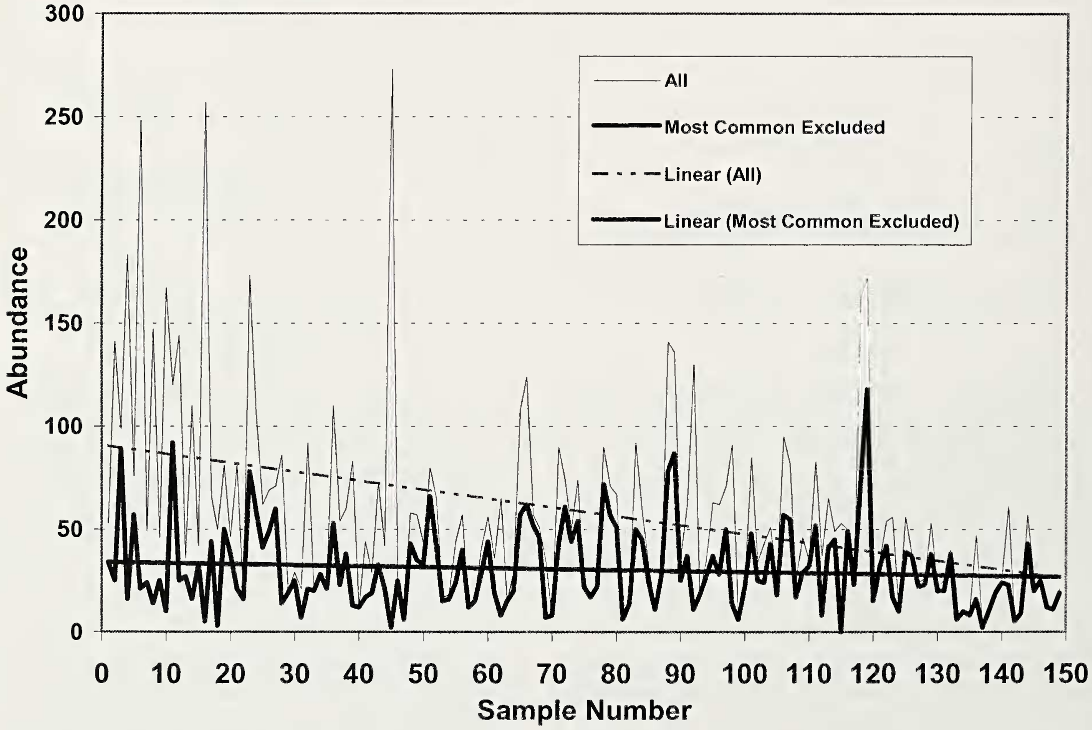


Figure 7.—Number of adults per chronologically arranged sample for all the data and with the two most common species removed, with least squares linear fits to each sequence.

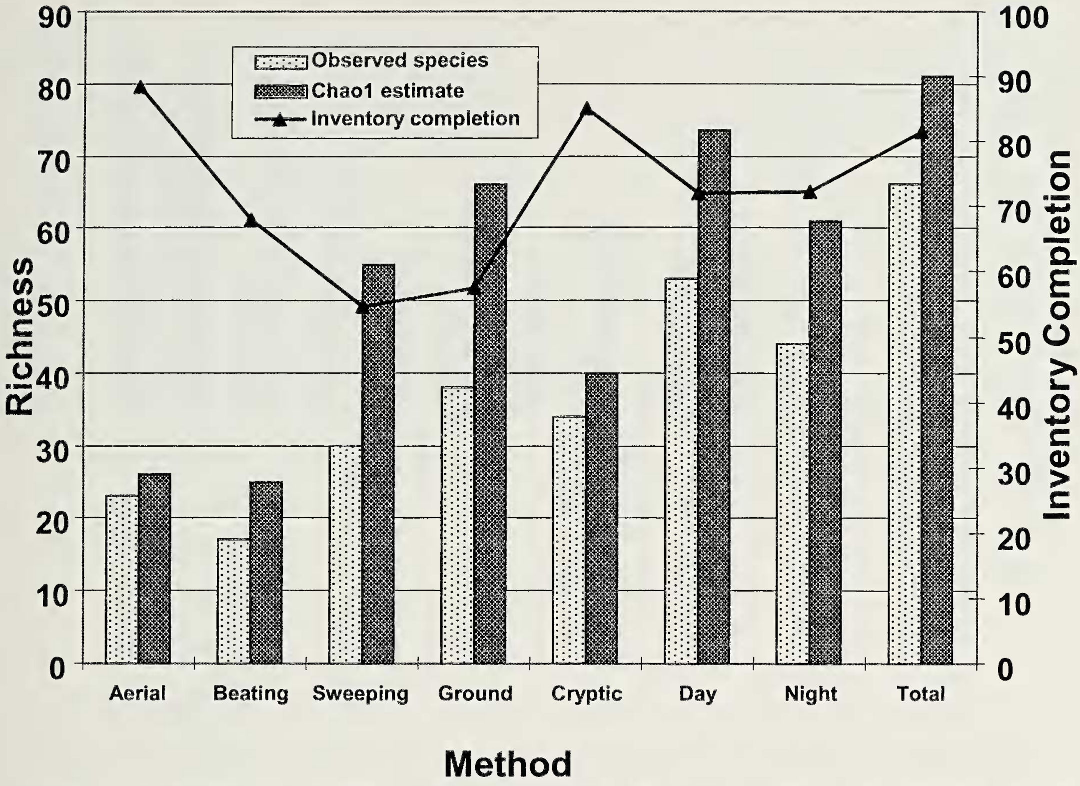


Figure 8.—Total species caught by each method and time of day, with Chao1 estimates and inventory completion values for each partition.

that differential investment does compensate for differential richness in habitats. Nevertheless, sweeping and ground faunas appear to have been relatively undersampled and aerial and cryptic faunas relatively oversampled compared to the mean inventory completion, so that this particular allocation profile mitigated, but did not eliminate bias due to differential return on effort by method. Richness plotted against individuals collected still shows positive slope and correlation (Fig. 9, "original"). If "return on effort" were saturated, the regression line would be essentially flat. Although the current inventory still shows a non-zero slope and correlation, less effort would have yielded an even steeper slope. Figure 9 also plots regressions for one third and two thirds of all samples randomly chosen from each method. One third as much effort shows a much steeper slope, and two thirds is intermediate between one third and the total data set, as expected (Fig. 9). Although substantial effort was invested in this inventory, it was nevertheless insufficient to eliminate correlation between sampling effort and observed richness.

Richness estimation.—The rank-abundance plot for the 66 observed species shows a characteristically temperate faunal distribution with relatively many common and few rare species compared to tropical faunas (Fig. 10). The ZMUC data fit a lognormal distribution (chi square goodness of fit, $P < 0.7$) but show no mode (Fig. 11, "ZMUC"). The richness estimation curves show typical signs of an incomplete inventory (Fig. 12): the observed curve terminates substantially below the estimator curves and is not asymptotic, the estimators are not consistently asymptotic, the uniques curve is still rising or barely flat, lies relatively far above the doubletons curve, and shows no sign of crossing it, and the doubletons curve is definitely still rising. At face value, the richness estimators presented here imply about 80–90 species present as adults in the area sampled and accessible to the methods used, of which we observed only 66 (73%).

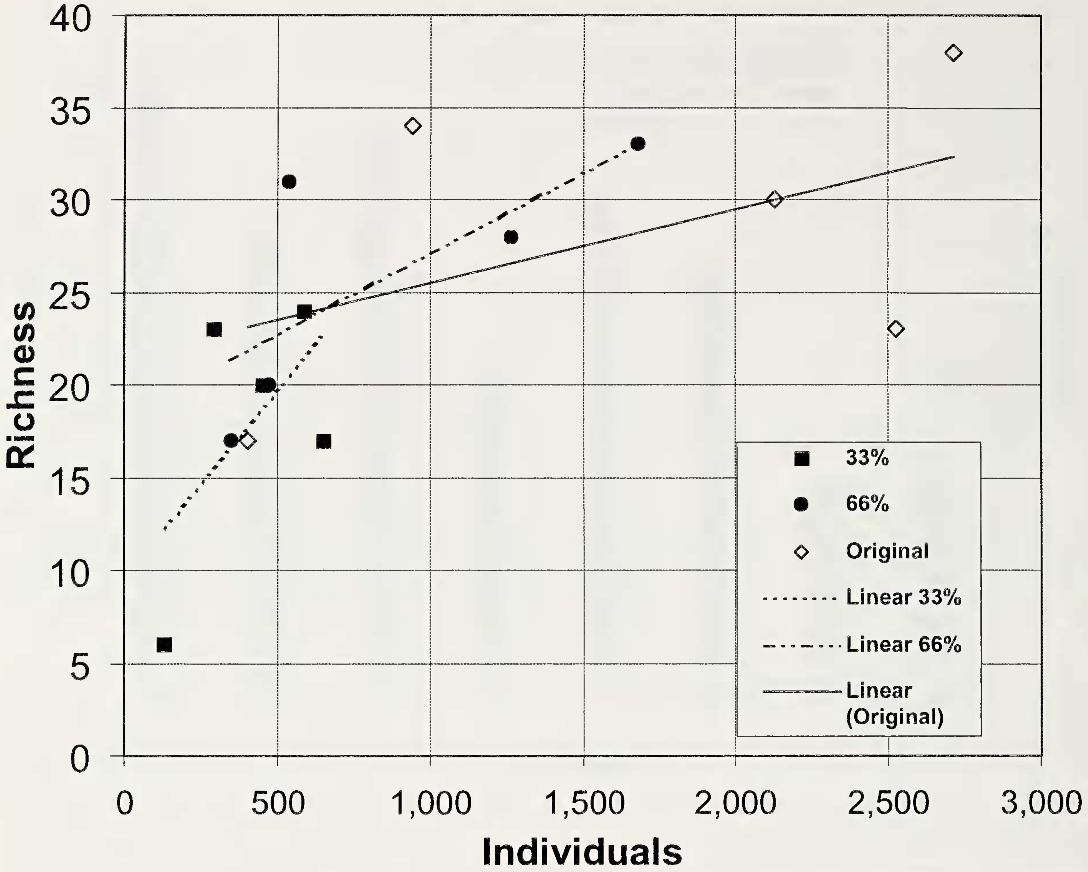


Figure 9.—The correlation between richness and sampling effort by method (measured as number of individuals sampled) for 33%, 66% and the total (original) dataset, with least squares fits to each partition.

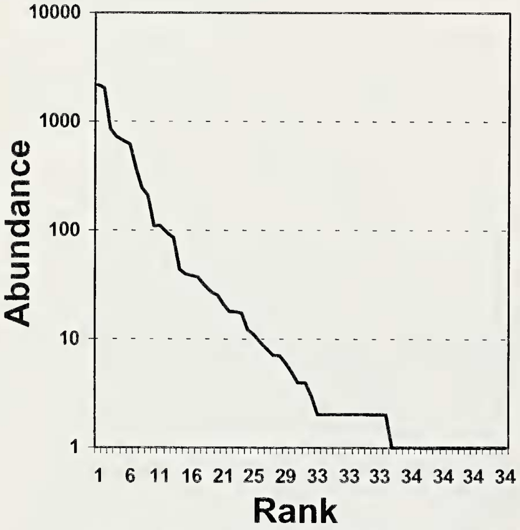


Figure 10.—Rank abundance diagram for the total dataset.

Comparison to AAU study.—Søren Toft sampled the same hectare as well as the surrounding beech forest on a roughly biweekly schedule from July 1969–July 1971 using five methods: litter extraction, pitfall and stem traps, sweeping and "clubs": a method in which trees are struck with very large clubs to dislodge the canopy fauna (Nielsen 1975; Toft 1976). He obtained 43,580 spiders of 147 species (plus 3 species that he could only assign to genus) over the two year study and classified them all to species and, if juvenile, to instar. The original AAU data sheets still exist and we used them to compile a database of species by instar, abundance and sample characteristics (i.e. date, method, etc.). Not surprisingly after 23 years, some discrepancies could not be resolved, but the database eventually accounted for 42,273 animals of 141 species, comprising 15,533 adults and 26,740 juveniles (Table 2). The missing spe-

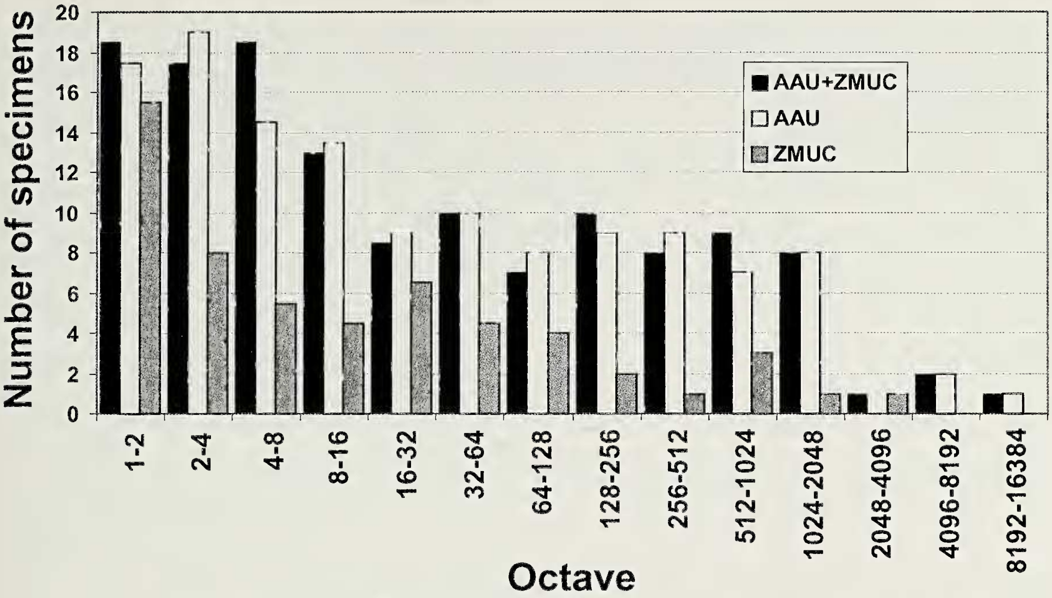


Figure 11.—Lognormal fits for the ZMUC and AAU data alone and combined.

cies and animals were mainly extremely small, unidentifiable juveniles that we excluded from the data. This unparalleled arachnological data set offers a unique opportunity to evaluate critically the more rapid and certainly less thorough ZMUC inventory at the same site. When pooled, the AAU 1969 and 1970 August and September collections total 2,260 adults. August alone comprised 47 species (11 singletons, 7 doubletons) and September 49 species (10 singletons, 12 doubletons); together the list comprised 57 species (16 singletons, 13 doubletons). Considering that for these two months Toft collected only about one fourth

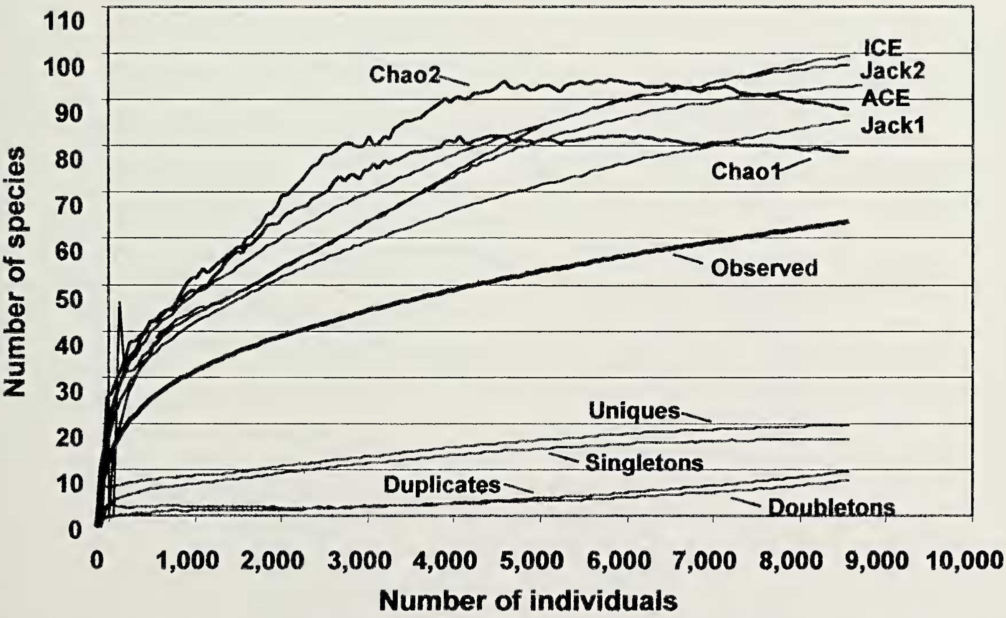


Figure 12.—Curves for observed richness, rare species, and richness estimators for the ZMUC inventory against sampling effort.

Table 2.—Summary values for the AAU inventory at Hestehaven. SD = standard deviation, Spp = species, A = adults, J = juveniles.

	Clubs		Stems		Sweeping		Litter		Pitfalls		Total		Grand Total
	A	J	A	J	A	J	A	J	A	J	A	J	
No. of samples	23		30		21		49		55		178		178
Mean no. of ind./sample	43	91	51	55	122	987	18	25	175	19	87	150	238
SD of ind./sample	36	90	77	57	119	1,134	15	27	201	19	140	492	526
Mean no. of spp./sample	18		10				11		20		17		17
SD spp./sample	7		4.9				3.5		9.7		10		10
Total individuals	988	2,102	1,519	1,644	2,562	20,726	862	1,240	9,602	1,028	15,533	26,740	42,273
Total species	48	39	44	29	60	52	33	37	82	48	130	79	141
Sample intensity	21	54	35	57	43	399	26	34	117	21	119	338	300
Singletons	16	7	14	4	20	6	7	12	20	9	32	11	27
Doubletons	6	2	10	6	9	1	4	0	2	3	12	1	8
% Singletons	33.3	17.9	31.8	13.8	33.3	11.5	21.2	32.4	24.4	18.8	24.6	13.9	19.1
Chao1 estimate	69	51	54	30	82	70	39	—	182	62	173	140	187
% Inventory Completion	69	76	82	96	73	74	84	—	45	78	75	57	76
% Method Bias	−7	0	6	20	−3	−2	8	—	−31	2	−1	−19	0
% Effort Investment	14		5		15		32		34		100		

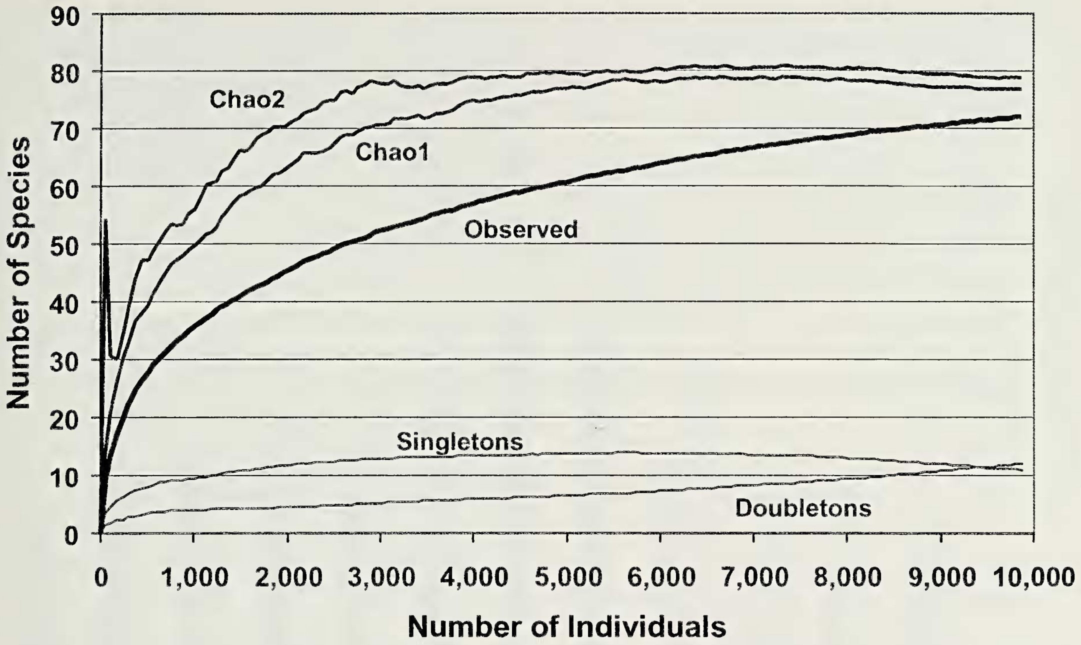


Figure 13.—Curves for observed richness, rare species, and richness estimators for the ZMUC and AAU data for the four week period centered on the ZMUC inventory.

as many adults as the ZMUC total of 8,710, his results are certainly comparable to our 66 species (19 singletons and 12 doubletons). The AAU August and September adult data contained 13 species not found in the ZMUC data, all but two singletons or doubletons, whereas the ZMUC study found 22 species not found by AAU for August–September, all but three singletons or doubletons. If the ZMUC list is compared to the total, annual AAU list, juveniles included, only five ZMUC species are missing from the AAU dataset.

The full two years of AAU data (including juveniles) considered separately and together with the ZMUC data yield a more complete lognormal distribution: the AAU data alone do show a mode one octave to the right of the ZMUC maximum (chi square goodness of fit, $P < 0.975$), and the two datasets combined (chi square goodness of fit, $P < 0.9$) place the mode even further to the right (Fig. 11).

The "best" estimate of the instantaneous richness during the ZMUC inventory is presumably that based on the maximum data available for the seasonal period and the methods used. The ZMUC inventory used neither pitfall traps nor "clubs" but sweeping and litter sifting were common to both studies, and stem traps are quite similar to aerial searching. Excluding the latter methods and taking into consideration annual seasonal variation, we selected all adults collected two weeks before and after the ZMUC sampling dates as the most complete data set for this time period (thus adding 1969–1970 AAU data to the ZMUC study) and calculated richness estimates using this dataset totaling 9,871 adults. Figure 13 shows these curves. The estimates appear substantially better than the ZMUC data alone: the estimator and observed curves are more asymptotic and closer together; the singleton and doubleton curves actually cross. The parametric richness of the adult spider fauna is suggested to be around 80 species.

The observed and Chao1 estimated species richness calculated for each month of the AAU dataset show the summer peak expected in a north temperate fauna (Fig. 14). August–September is substantially past the annual May–June richness peak, judged either by observed or estimated richness. Comparison of estimated to observed richness for the AAU study shows that the level of sampling effort was relatively better early in the year, insufficient to keep up with the May–June peak, recovered somewhat in August–September, and fell off again in October–December.

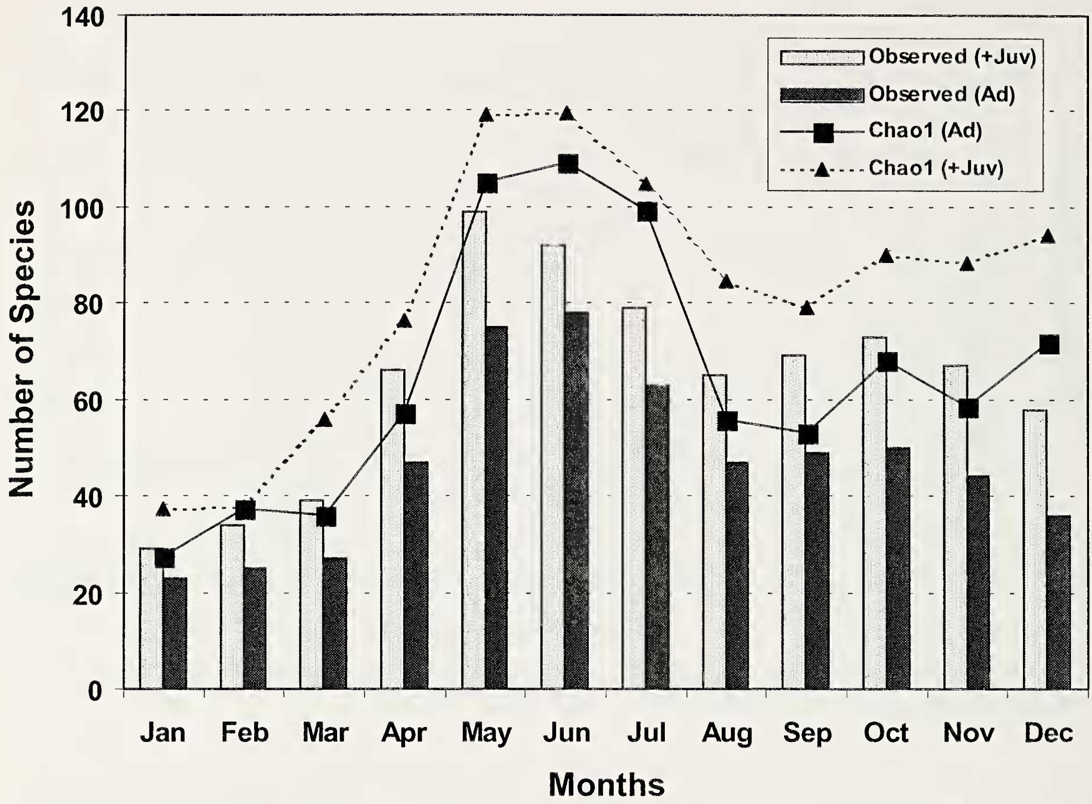


Figure 14.—Total species observed (adults only and including juveniles) for each month of the AAU inventory, with Chao1 estimates based on both partitions.

Monthly-observed adult richness varied from 23 in January to 78 species in June.

Despite the large number of animals collected, the ZMUC inventory still contained 19 singletons and 12 doubletons. The null hypothesis of richness estimation is that rare species indicate undersampling. However, there are at least three alternative explanations for "rare" species besides undersampling bias. Conceptually these are all "edge effects" due to time, method, or space (Longino et al. 2002).

Phenological edge effects.—A phenological edge effect is an individual that is mature outside the normal breeding season of its species. The AAU study aimed principally to reconstruct the life history and phenologies of the spider community at this site. These data (Fig. 15) can be used to "diagnose" which of the rare ZMUC species are "phenological edge effects." For example, 48 of the ZMUC species are normally adult at the time of the inventory, but 13 are typically adult at other times: 12 earlier and one later. We counted a species as a phenological edge effect (as opposed to just being rare) if its total abundance in the AAU study was more than 10, and the time span of adults did not include August or September. Of the 19 singleton and 12 doubleton species in the ZMUC inventory, eight singletons (*Anyphaena accentuata* (Walckenaer 1802), *Araniella curcubitina* (Clerck 1757), *Hypomma cornutum* (Blackwall 1833), *Linyphia hortensis* Sundevall 1830, *Micrargus herbigradus* (Blackwall 1864), *Neriene peltata* (Wider 1834), *Walckenaeria obtusa* (Blackwall 1836), *Pachygnatha listeri* Sundevall 1830) and two doubletons (*Diplocephalus latifrons* (O. P.-Cambridge 1863), *Saaristoa abnormis* (Blackwall 1841)) were out of season and arguably are not evidence of undersampling bias.

Methodological edge effects.—A method edge effect is an individual of a species that typically inhabits a microhabitat not accessed by any of the methods used, or, at least, not efficiently accessed. If a singleton or doubleton ZMUC species was commonly collected in the AAU study by a method not used in the ZMUC study (i.e. pitfalls or clubs), it is arguably a methodological edge effect and not evidence of undersampling. As above, if the total AAU abundance was more than ten and mainly caught via pitfalls or clubbing, we count it as a methodological edge effect. *Neriene peltata*, *Achaearanea lunata*, and especially *A. accentuata* were all substantially more common in the canopy than in subcanopy strata. Some also showed minor peaks in abundance in sweep samples, suggesting that rarely animals may fall or jump from the canopy and so appear in the herb layer. *Walckenaeria obtusa*, *M. herbigradus*, *P. listeri*, *D. tibiale*, *Lepthyphantes cristatus*, *L. pallidus*, *S. abnormis*, and *D. latifrons* were taken almost exclusively by pitfall traps, although the latter also appeared in litter samples. These 11 species are probably rare in the ZMUC study because they are accessible mainly via methods we omitted, although five were also out of season.

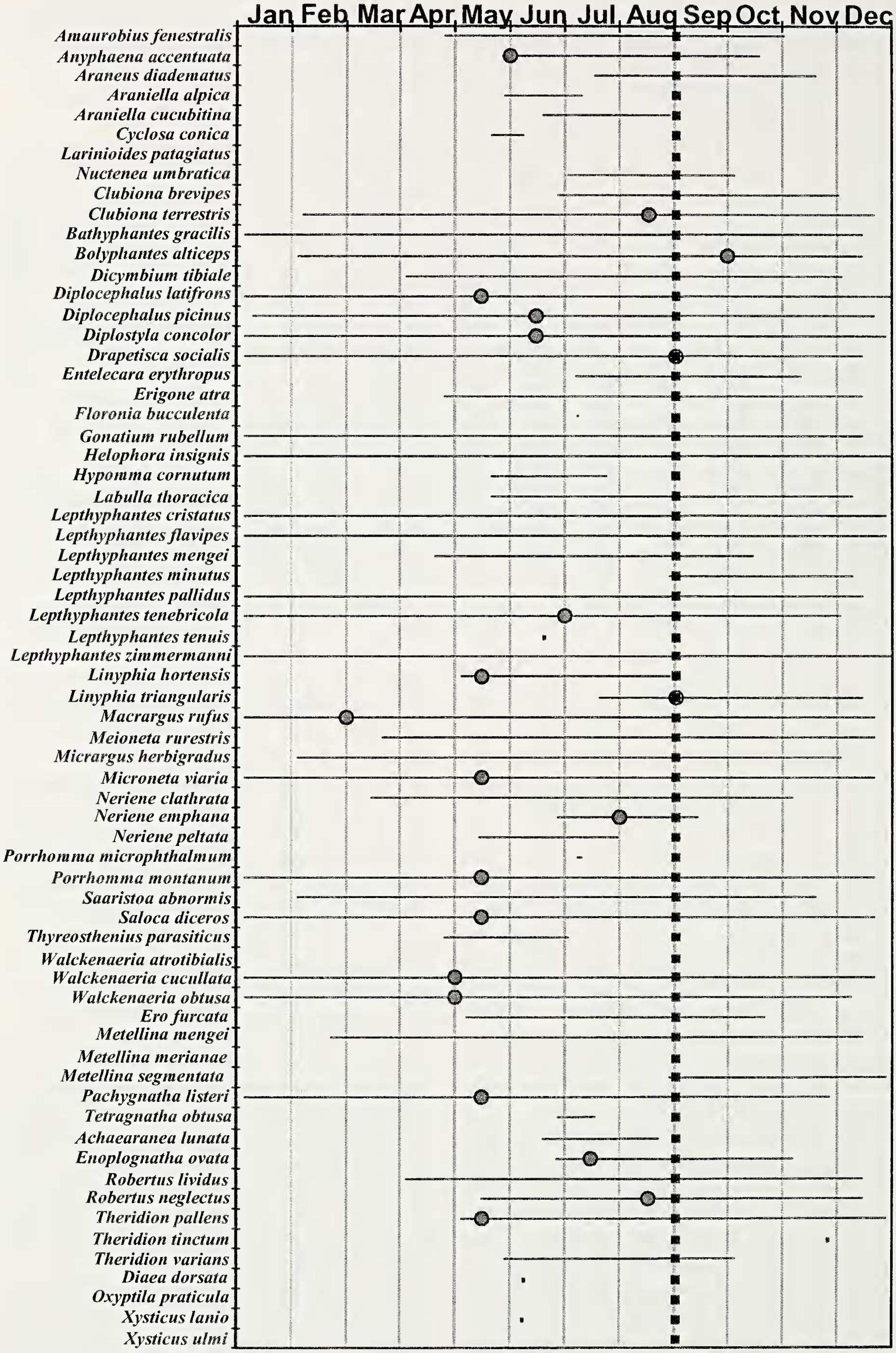
Spatial (habitat) edge effects.—A spatial edge effect is an individual of a species that prefers a habitat not present in the study area. The hectare was fairly uniform, but it had a wet depression at its lower end. *Tetragnatha obtusa* C.L. Koch 1837 may have been rare in both studies because it prefers wetter situations and thus barely enters the plot. Arguably it is not evidence of undersampling. Although not present in the ZMUC study, *Hyptiotes paradoxus* (C.L. Koch 1834) was rare in the AAU study; it prefers the coniferous plantations adjacent to the study hectare and may have been sporadically and unreliably present within study margins. *Metellina merianae* (Scopoli 1763), *Erigone atra* Blackwall 1833 and *Nuctenea umbratica* (Clerck 1757) were rare in both studies, suggesting they may be typical of habitats other than mature beech forest. No Hestehaven "rare" species are truly rare in Denmark.

In sum, of the 19 singleton and 12 doubleton ZMUC species, nine singletons and three doubletons are rare due to edge effects and should not be considered as evidence of undersampling. If these species are excluded from the inventory, and richness estimates recalculated (Fig. 16), the quality of the inventory improves substantially. The estimator curves are definitely asymptotic (at about 4,000 sample size), the observed curve still trails the estimator curve, and the uniques curve is almost flat, and the duplicates curve, unusually, goes to zero.

DISCUSSION

How many species of spiders typically inhabit one hectare of northern European climax beech forest? How much effort is required to answer the question or estimate that number, or how would one know when an observation or estimate was accurate? These questions make sense only if assumptions about temporal and spatial scales are made explicit. The minimum realistic spatial scale that is biologically real is one large enough to include demes of all resident species, species-area effects aside. Biparental organisms, in other words, should be present at least in abundances of two, and for all practical purposes many more. The latter reasoning provides a strong common sense justification for the Chao estimators of species richness, as they trade on the ratio of singletons (biological non-sequiturs) to doubles to correct for undersampling bias. Species literally present in a hectare as singletons don't make biological sense because they can't reproduce and must represent long-distance dispersal; doubletons, for all practical purposes, are the same. Of course, many animals live at spatial scales larger than a hectare, but for spiders, one hectare (100 × 100 m) seems like a reasonable minimum spatial scale because it is unlikely that the breeding population structure of spiders, i.e. the "nearest-neighbor distance," is so dispersed that single hectares are likely to contain one or fewer individuals. A spatial scale of one meter might be appropriate for litter fauna but inappropriate for large cursorial hunters or web weavers. For the latter, as a guess, even 10 m seems excessive. At larger spatial scales the species-area effect will be increasingly important. The Danish national checklist currently stands at 500 species (Scharff 1984). Checklists overestimate current standing diversity because they are cumulative, and not corrected for faunal turnover. Thus, the "instantaneous," ecologically meaningful, richness of spider species in Denmark is probably less.

The "checklist" of Hestehaven listed by Toft (1976) includes 147 identified species. Twenty-three years later the ZMUC study added only five more (*M. merianae* (Scopoli


Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Amaurobius fenestralis
Anyphaena accentuata
Araneus diadematus
Araniella alpica
Araniella cucubitina
Cyclosa conica
Larinioides patagiatus
Nuctenea umbratica
Clubiona brevipes
Clubiona terrestris
Bathyphantes gracilis
Bolyphantes alticeps
Dicymbium tibiale
Diplocephalus latifrons
Diplocephalus picinus
Diplostyla concolor
Drapetisca socialis
Entelecara erythropus
Erigone atra
Floronia bucculenta
Gonatium rubellum
Helophora insignis
Hypomma cornutum
Labulla thoracica
Lepthyphantes cristatus
Lepthyphantes flavipes
Lepthyphantes mengei
Lepthyphantes minutus
Lepthyphantes pallidus
Lepthyphantes tenebricola
Lepthyphantes tenuis
Lepthyphantes zimmermanni
Linyphia hortensis
Linyphia triangularis
Macrargus rufus
Meioneta rurestris
Micrargus herbigradus
Microneta viaria
Neriene clathrata
Neriene emphana
Neriene peltata
Porrhomma microphthalmum
Porrhomma montanum
Saaristoa abnormis
Saloca diceros
Thyreosthenius parasiticus
Walckenaeria atrotibialis
Walckenaeria cucullata
Walckenaeria obtusa
Ero furcata
Metellina mengei
Metellina merianae
Metellina segmentata
Pachygnatha listeri
Tetragnatha obtusa
Achaearanea lunata
Enoplognatha ovata
Robertus lividus
Robertus neglectus
Theridion pallens
Theridion tinctum
Theridion varians
Diaea dorsata
Oxyptila praticula
Xysticus lanio
Xysticus ulmi

1763) , *Larinioides patagiatus* (Clerck 1757), *Ozyptila praticola* (C.L.Koch 1837), *Walckenaeria atrotibialis* (O. P.-Cambridge 1878), *Xysticus ulmi* (Hahn 1831)), all singletons or doubletons except *L. patagiatus* (7 individuals). The absence of faunal additions in 23 years is impressive (we cannot comment on losses): 92% of the ZMUC species were shared with the AAU study. None of the species added by the ZMUC study were present at a relative abundance of more than 0.0008, which also suggests that the fauna is stable over time.

"Instantaneous" Hestehaven richness is much less than 147 species, at least during August–September. The monthly adult richness observed by Toft (1976) ranged from a January low of 23 to a June high of 78 (Fig. 14) and averages 47 ± 18 (sd); the August–September values were 47 and 49. These figures do not include species present as juveniles, which comprised 63% of the spider community in Toft's study. If juveniles are included, monthly richness varies from a January low of 29 to a May high of 99, and averages 64 ± 22 (sd); the August–September values were 65 and 69. Although the average AAU monthly sampling intensity for adults was only 24, the average monthly percent singletons was 27%, essentially the same as in the much more intense 2.5 day ZMUC effort (29%). Although this small sampling effort seems to provide the same percent singletons as the much more intense ZMUC study, our experience is that even small decreases in percent singletons demand logarithmic increases in effort. All the AAU figures still suffer from undersampling bias. The best bias-corrected figures we have are the adult-only estimates for August–September provided by the combined AAU-ZMUC data, which is about 75–80 species (Fig. 13). This figure, then, is predicted to be the ballpark adult spider richness a complete survey would find for this season in this forest using the methods of the ZMUC inventory.

Chao1 estimates of monthly richness from the AAU study, including juveniles, range from a January low of 37 to a June high of 120; August and September values are 85 and 79, respectively (Fig. 14). Because these figures include juveniles, phenological edge effects are minimized, in which case remaining possible biases are method and spatial edge effects. *Hyptiotes paradoxus* is perhaps the only undeniable example of the latter, a species which "should not have been" in the Hestehaven beech wood. The spectrum of methods used by Toft accessed all substrates used by spiders except the high canopy. Both the intense sampling of the ZMUC survey and the effort to identify juveniles by the AAU survey yield essentially the same estimates: the per-hectare August–September standing spider species richness at Hestehaven is probably about 80 species.

If the above is true, the Hestehaven checklist richness of 150 species at first seems paradoxical. If the greatest monthly observed richness is only about 120 species, juveniles included (Fig. 14), where are the remaining 30 species? The most obvious explanation is, again, undersampling bias in the AAU study. Even though Toft identified every animal collected to species, the substantial fraction of singletons in all AAU partitions argues that he missed quite a few species. However, two other explanations should be considered. First, the missing species may not be permanent year-round residents in the sampled hectare. This implies considerable flux of species such that the standing richness indeed fluctuates between about 40 and 120 species, which in turn poses the question of where these species go. As the many hectares of forest adjacent to the study area were essentially identical, mass migration seems unlikely. Second, if the 30 species do remain in the hectare, why is the observed richness not more consistent from month to month? Only two possible explanations seem likely. First, they may vertically migrate into canopy strata that neither inventory accessed. Nielsen (1974c, 1987) found that portions of the arthropod fauna do migrate up and down at this site several times a year. Second, for some portion of the year

←

Figure 15.—Phenology for the 66 species observed in the ZMUC inventory, based on AAU data. Thin horizontal lines give the range during which adults were found (gaps not indicated). Grey indicate peak adult abundances, if present. Squares mark the ZMUC inventory.

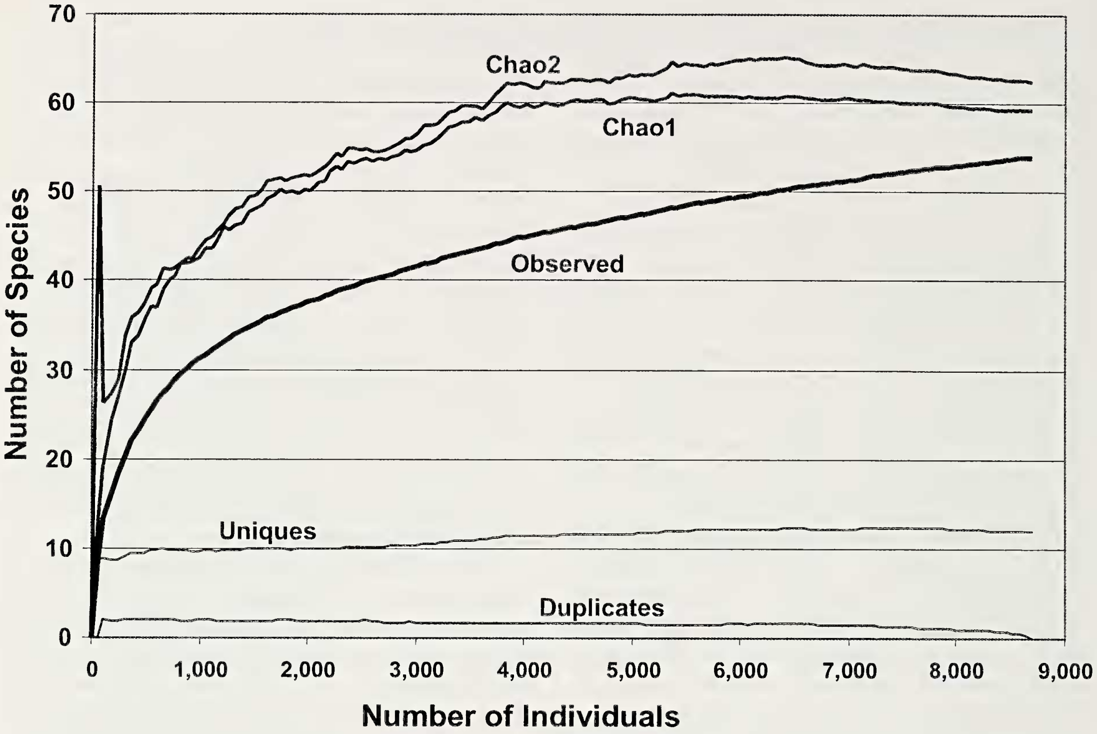


Figure 16.—Curves for observed richness, rare species, and richness estimators for the ZMUC data pruned of artifactually rare species (see text).

these species certainly exist only as eggs or may hide in retreats not accessible to the collecting methods. Thus, the ecologically meaningful late summer spider fauna is probably close to the Chao estimates in Fig. 13—about 80 species.

The AAU and ZMUC studies are best compared on the basis of adults only. For the same time period the AAU sampling intensity was much lower than the ZMUC study (27 vs. 132), but percent singletons was roughly comparable (22 vs. 29%). In terms of specimens collected, the ZMUC study was five times more intense than the AAU effort. The extra ZMUC effort netted about 20 more species, of which 18 were singletons or doubletons—exactly the sort of species the less intense AAU sampling effort would be expected to miss.

Both Figs. 13 & 14 suggest that the ZMUC inventory sampled more spiders than necessary to estimate richness. Perhaps 3–4,000 adults sampled would have been sufficient to estimate species richness, given that the rank-abundance distribution was heavily skewed towards a few extremely abundant species. This depends on collectors being able to recognize accurately the very abundant species in the field.

The heavy reliance on hand-searching during the ZMUC study did not yield significant numbers of species that were missed by the AAU study's reliance on methods less dependent on collector experience. Collector experience does significantly improve the number of species taken per sample, and, usually, experienced collectors do not differ among themselves (see also Sørensen et al. 2002). The collector in Fig. 4 that was classified a priori as experienced did not actually have any experience with this sort of sampling, although he had reportedly collected spiders for many years. How much time a naïve collector requires to become "experienced" is still an open question. Collector experience has only a minor and insignificant effect on numbers of animals. In particular, the maximum number of animals caught per hour (273) greatly exceeds the average (58), which means that observed sample abundances are not limited simply by how fast collectors can collect. Granted that that human collectors as a sampling method will have its own intrinsic bias (as do all sampling methods), variation in observed abundances probably does reflect gross differences in true relative abundance in the field. Experienced collectors, at any rate, do not catch more species because they catch more animals; the reason is probably that they know more places to look in order to find spiders.

The extreme ecological dominance of *L. triangularis* and *D. socialis* made this inventory less complete than it otherwise would have been. Nearly 50% of the animals collected—quite a practical measure of sampling (and sorting) effort—disappeared into the arguably useless exercise of collecting superfluous specimens. In fact these two species illustrate the extremes of the effects that extreme ecological dominance can have. After one night we truncated collection of *D. socialis* because it was very abundant and easily recognizable. Human collectors can do this. If we had used automated ecological traps, a very great many more *D. socialis* would have died. At the other extreme, two rare species look enough like *L. triangularis* in the field that one cannot reliably distinguish them. Therefore we continued to collect "*L. triangularis*," and eventually collected one *L. hortensis* late in the survey. We sacrificed accuracy of the relative abundance of common species to focus on rare species and to moderate our effect on the fauna. Still, the superabundance of a few species may make it hard for collectors to collect the remainder in an unbiased way. The most abundant spider species in tropical ecosystems rarely exceed 15% of the total (Coddington et al. 1991, 1996; Silva & Coddington 1996; Silva 1996), and that seems mainly to occur at high elevations (Sørensen et al. 2002). Very common species actually may make temperate ecosystems more difficult to survey in some ways than tropical systems.

As expected, collection method and time of day also influence results (Figs. 5–6). Not only are some methods more productive, all methods seem to access different sampling universes (Table 1), which justifies the broadest possible spectrum of collecting methods in faunal inventories that aim to be complete.

Sampling methods access different components of the fauna. Equal effort among methods implicitly assumes that all methods are equally efficient, and that the sampling universes particular to each method are roughly the same size. These assumptions are clearly unrealistic, and thus to minimize sampling bias, inventories should differentially allocate effort among methods, if a goal of the inventory is to sample the community with as little bias as possible. We suggest that inventory completion is a reasonable, albeit imperfect, statistic to measure this bias. It implies that the optimal allocation strategy would yield similar inventory completion measures for all inventory partitions, whether by method, time of day, or other partitions. Thus, all partitions might be undersampled, but they would be, in some sense, "equally" under-sampled. The ZMUC study emphasized cryptic and ground searching in anticipation of large numbers of ground-dwelling linyphiid species. The results suggest that the cryptic fauna was relatively over-sampled, and the ground fauna relatively undersampled, which in turn suggests that the sample of the overall spider community we obtained is biased in particular ways, although not as much as it would have been had the sampling allocation been more nearly uniform. One could, for example, calculate richness for various taxa and assess how well the methods sampled those taxa. If the sampling regime had lasted more days, litter and pitfall samples could have been added without diminishing the amount of time for collector-based sampling. We certainly support using as many techniques as resources permit. Allocation of sampling effort across methods is a serious problem. Although ideally the sample should reflect the parametric community, and in theory richness estimators should identify departures from that ideal, we do not know if the inventory completion statistics are robust from one study or region to another. If a given investment in, say, sweeping, produces wildly different and unpredictable results from place to place, year-to-year in the same place, or study-to-study, it will not be a useful analytical technique.

Grossly different numbers of samples between methods or times of day inevitably produce highly unbalanced statistical designs for analysis of variance. However, the natural history logic of investing more in productive as opposed to less productive methods in our view outweighs the analytical convenience of a completely balanced design. First, the statistical differences we detected in this study are large ($P < 0.00$) and are unlikely to disappear in a balanced design. Second, modern statistical packages can correct much better for unbalanced designs than formerly. Third, if necessary one can include only the first N samples in each analysis of variance cell, where N is the global minimum cell size. This provides an unambiguous way to test sampling effects while still freeing the investigator to allocate sampling effort in the way best calculated to access efficiently and accurately the total fauna.

This study demonstrates design and analytical methods by which undersampling bias in terrestrial arthropod surveys can be detected and measured. The evidence for severe undersampling bias in arthropod surveys is pervasive if measured by percent singletons. Large samples do not indicate a thorough inventory if the inventory scope was broad. In spiders, for example, the fogging of the canopy of a single tree from Manu National Park, Perú by T. L. Erwin yielded 222 adult spiders of 124 species, 63% of which were singletons, and multiple tree canopies from Tambopata, Peru, yielded 1,821 adult spiders of 645 species, 55% of which were singletons (Coddington, unpublished data). Silva (1996) reported 43% singletons in a collection of 5,895 adults of 1,140 species from Samiria, Peru, collected mostly by fogging. A recent spider canopy study from Tanzania had 23% singletons (Sørenson 2003). Other authors often report diversity statistics for fogging samples rather than raw numbers, but because Fisher's alpha approximates the number of singletons, Russell-Smith and Stork (1994) must have found an average of about 45% singleton spider species in fogging samples at four stations along an elevational transect in Sulawesi. Subcanopy manual collecting in Manu yielded 2,616 adults of 498 species with 42% singletons (Silva and Coddington 1996). Three points along an elevational transect in Bolivia averaged 44% singletons in subcanopy faunas (Coddington et al. 1991, 1996). Kuntner and Baxter (1997) found 54% singletons in subcanopy collections in Slovenia. Singleton percentages for spider inventories are not out of line with terrestrial arthropods generally. Novotny and Basset (2000) collected over 80,000 homopterans, but these comprised over 1,000 species, of which 27% were singletons. Toft (1976) and this study together collected 50,983 spiders of 146 species, but again 27% were singletons. Basset et al. (1996) collected 4,696 individuals of 391 species of beetles, and percent singletons was 39% (Basset 1997). Basset and Kitching (1991) collected 20,500 individuals of 759 subcanopy and canopy species but 36% were singletons. The canopy fraction was higher at 45%; among spiders it was 42%. Allison et al. (1997) sampled 3,977 individuals of 481 species of beetles, but 46% were singletons. Erwin (1997) reports collecting 15,869 Peruvian beetles of 3,429 species of which 50% were singletons. Janzen and Schoener (1968) reported 65% singletons in their arthropod collections from all of Costa Rica, and Noyes (1989) reported 60% in Chalcidoidea from Sulawesi. Monteith and Davies (1984) likewise found 40% singletons during a month-long survey of Queensland rainforest. In relative abundance distributions such as these doubletons are very probably about half the singletons, so something like 50–70% of the species found in many "state of the art" arthropod surveys are known from two or fewer individuals.

This study was able to explain only about a third of the rare species as artifacts of one sort or another. The remainders imply that even after intense sampling, observed richness understated true richness by at least 20%. The relative abundances of the species found by ZMUC and missed by AAU for August–September is consistent with the hypothesis that most "rare" species (singletons) in terrestrial arthropod surveys are legitimate members of the community. The tendency to ignore rare species as "tourists" should be viewed with skepticism (Stork & Samways 1995). These estimates formally are all lower bounds (Bunge & Fitzpatrick 1993; Colwell & Coddington 1994), so the actual situation is probably worse. Figures 12, 13 and 16 show that in practice estimators' asymptote only when about two-thirds or more of the species are already observed. In sum, both statistical species richness estimators and the observed richness are negatively biased with respect to parametric community richness for most of the time course of an inventory. Richness estimates statistically corrected for undersampling bias are nevertheless more accurate than the raw, observed richness, and, depending on the degree of accuracy required, probably almost never show significant positive bias in practice.

Because return-on-effort in inventories is inevitably curvilinear, direct comparisons of richness values between sites are likely to be fraught with bias and error (Gotelli & Colwell 2001). This study also shows that for the first 2,000 or so specimens as a measure of effort, even the most aggressive richness estimators are still strongly and negatively biased. Considering that a sample of 2,000 animals from a parametric community richness of about 80 species still represents a sampling intensity of 25, and that sampling intensities of less than 10 are probably typical of most work, one must question the prevailing paradigm of spreading arthropod inventory resources as thinly as possible in pursuit of broad goals and diverse taxa. It is the rare terrestrial arthropod inventory taxon that does not have twice, or even 10 times the anticipated diversity as any sympatric vertebrate group, and arthropod surveys generally make do with less resource than vertebrate surveys. Masters theses that envisage a single student sampling a diverse taxon once or twice a month over an annual cycle in a seasonal environment is almost certain to result in data so sparse that absence due to undersampling bias will be indistinguishable from that due to biologically interesting variation (McArdle & Gaston 1993). Of course, not all surveys aim to measure or estimate richness, but comparative species richness is increasingly the most important datum, at least initially, in biodiversity conservation (Mittermeier et al. 1998). Nevertheless, until very recently, manuals and treatments of inventory methods rarely mention undersampling bias (Hayek & Buzas 1997; Stork & Samways 1995), but see (Leitner & Turner 2001). Compared to the initial costs of mounting the survey to begin with, designing and funding it well enough to secure verifiably reliable data seems at most a marginal cost increase. If the conservation of biodiversity depends on reliable data, both funding agencies and the designers of inventory protocols should reconsider prevailing practices.

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Appendix 1.—Species and number of adult spiders collected in Hestehaven, Denmark; Collection method and time of day indicated by: D = (Day; N = Night); Nomenclature following Platnick (2002).

	Collection method										
	Aerial		Beating		Cryptic		Ground		Sweeping		
Taxon	D	N	D	N	D	N	D	N	D	N	Total
Anyphaenidae											
Anyphaena accentuata (Walckenaer 1802)				1							1
Amaurobiidae											
Amaurobius fenestralis (Stroem 1768)		5			23	2	5	8		1	44
Araneidae											
Araneus diadematus Clerck 1757	6	35	6			1	3	4	18	12	85
Araniella alpica (L. Koch 1869)									1		1
Araniella cucurbitina (Clerck 1757)		1									1
Cyclosa conica (Pallas 1772)							1		1		2
Larinioides patagiatus (Clerck 1757)		4						1		2	7
Nuctenea umbratica (Clerck 1757)										1	1
Clubionidae											
Clubiona brevipes Blackwall 1841			1	6				3		1	11
Clubiona terrestris Westring 1851		3	7		209	7	148	190	12	92	668
Linyphiidae											
Bathyphantes gracilis (Blackwall 1841)									4		4
Bolyphantes alticeps (Sundevall 1833)					7	2	18	5	4	4	40
Dicymbium tibiale (Blackwall 1836)					2						2
Diplocephalus latifrons (O.P.-Cambridge 1863)					2						2
Diplocephalus picinus (Blackwall 1841)					15	1	3	1		1	21
Diplostyla concolor (Wider 1834)					14			3			17
Drapetisca socialis (Sundevall 1833)	204	1501	25	2	29		95	172	11	7	2046
Entelecara erythropus (Westring 1851)					1						1
Erigone atra Blackwall 1833									2		2
Floronia bucculenta (Clerck 1757)								2			2
Gonatium rubellum (Blackwall 1841)			1		11		6	4	2	1	25
Helophora insignis (Blackwall 1841)	9	21	139	39	80	8	111	60	188	193	848

Appendix 1.—Continued.

	Collection method										
	Aerial		Beating		Cryptic		Ground		Sweeping		
Taxon	D	N	D	N	D	N	D	N	D	N	Total
Hypomma cornutum (Blackwall 1833)										1	1
Labulla thoracica (Wider 1834)	1	40	4		53	9	231	391	2	3	734
Lepthyphantes cristatus (Menge 1866)							1	1			2
Lepthyphantes flavipes (Blackwall 1854)					56	2	82	90	8	9	247
Lepthyphantes mengei Kulczinski 1887								4			4
Lepthyphantes minutus (Blackwall 1833)		3					4	31			38
Lepthyphantes pallidus (O.P.-Cambridge 1871)					2						2
Lepthyphantes tenebricola (Wider 1834)					12		23	74			109
Lepthyphantes tenuis (Blackwall 1852)								1	3	4	8
Lepthyphantes zimmermanni Bertkau 1890		2	2	3	81	9	127	115	8	23	370
Linyphia hortensis Sundevall 1830							1				1
Linyphia triangularis (Clerck 1757)	110	440	91	11	55	2	303	228	536	359	2135
Macrargus rufus (Wider 1834)					13		3	2			18
Meioneta rurestris (C.L. Koch 1836)									7		7
Micrargus herbigradus (Blackwall 1854)					1						1
Microneta viaria (Blackwall 1841)					95	7	8	1			111
Neriene clathrata (Sundevall 1830)					1		1				2
Neriene emphana (Walckenaer 1841)		8	1								9
Neriene peltata (Wider 1834)		1									1
Porrhomma microphthalmum (O.P.-Cambridge 1871)								1			1
Porrhomma montanum Jackson 1913					6						6
Saaristoa abnormis (Blackwall 1841)					2						2
Saloca diceros (O.P.-Cambridge 1871)	4				14						18
Thyreosthenius parasiticus (Westring 1851)					26		1				27
Walckenaeria atrotibialis O.P.-Cambridge 1878							1				1
Walckenaeria cucullata (C.L. Koch 1836)	2				27	1	1	1			32
Walckenaeria obtusa Blackwall 1836					1						1
Mimetidae											
Ero furcata (Villers 1789)					1					1	1

Appendix 1.—Continued.

Taxon	Collection method										Total
	Aerial		Beating		Cryptic		Ground		Sweeping		
	D	N	D	N	D	N	D	N	D	N	
Tetragnathidae											
Metellina mengei (Blackwall 1869)	3	7	5				5	9	45	19	93
Metellina merianae (Scopoli 1763)					1			1			2
Metellina segmentata (Clerck 1757)	19	81	25	12	20	1	39	73	196	156	622
Pachygnatha listeri Sundevall 1830							1				1
Tetragnatha obtusa C.L. Koch 1837										1	1
Theridiidae											
Achaearanea lunata (Clerck 1757)		1						1			2
Enoplognatha ovata (Clerck 1757)		1							1	3	5
Paidiscura pallens (Blackwall 1834)	4	5	10		3				10	5	37
Robertus lividus (Blackwall 1836)	2				9	1					12
Robertus neglectus (O.P.-Cambridge 1871)			12		15		3	9	92	75	206
Theridion tinctum (Walckenaer 1802)		1	1								2
Theridion varians Hahn 1833		2	1								3
Thomisidae											
Diaea dorsata (Fabricius 1777)										1	1
Ozyptila praticula (C.L. Koch 1837)							1				1
Xysticus lanio (C.L. Koch 1835)							1				1
Xysticus ulmi (Hahn 1831)										1	1
Grand Total	364	2162	331	74	887	53	1227	1486	1151	975	8710